(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

B42

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 March 2003 (20.03.2003)

PCT

(10) International Publication Number
WO 03/022260 A1

(51) International Patent Classification[7]: A61K 31/195, 31/519, 9/08, A61P 35/00, A61K 31/505 // (A61K 31/519, 31:195)

(21) International Application Number: PCT/YU02/00017

(22) International Filing Date:
10 September 2002 (10.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
P-657/01 12 September 2001 (12.09.2001) YU

(71) Applicants and
(72) Inventors: **ARSENIJEVIC, Slobodan** [YU/YU]; Mite Cekica 22, YU-Kragujevac (YU). **MATOVIC, Zoran** [YU/YU]; Kragujevackog oktobra 145/11, YU-Kragujevac (YU).

(74) Agent: **KOJIC, Dragomir, M.**; Kneza Milosa 43, YU-Belgrade (YU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

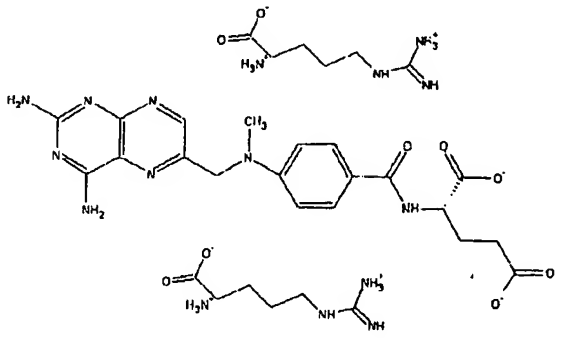
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*

[Continued on next page]

(54) Title: USE OF METHOTREXATE AND L-ARGININE FOR THE PREPARATION OF A MEDICAMENT FOR TREATMENT OF UTERINE MYOMA



(57) Abstract: The invention illustrates a drug for treatment of myoma of the uterus that enables preservation of biological and sexual properties of the women and does not provoke contraindications accompanying other known methods of treatment. The invention is represented by a mixture of two substances methotrexate and L-arginine. The drug for treatment of myoma of the uterus is composed of 5 g methotrexate and 10 g L-arginine with 985 cm^3 water. Combination of two substances in aqueous solution yields a homogenous mixture where each of the components preserves its own chemical identity. The drug is applied on the eighth day of the follicular phase of the menstrual cycle of patients with myoma of the uterus. The patient is placed into lythotomy position, the vaginal speculum is placed and the uterine cervix is pulled with roller forceps so that the external uterine cervix is exposed. The package is open, the puncture needle iS inserted into the cervical channel and is directed to the myometrium towards the side at which myoma is located. The needle is pushed through the endometrium to the depth of 1 cm. It is necessary to be sure to inject the drug at 1 cm depth to avoid possible penetration through the uterine wall. We aspirate to check that the needle is not inside a blood vessel, and after that inject the drug slowly. The syringe and needle are discarded after use. The mixture is applied in three consecutive menstrual cycles. The method is simple, feasible on out-patient basis by practicing gynecologist; no ultrasonografic guidance is needed; transvaginal and transcervical approach is used. The mixture induces the process of apoptosis (programmed cell death) so that no necrosis, degeneration or inflammation are provoked if all antisepsis precautionary measures are applied. Dramatic reduction of both loss of blood during menstruation in women with myoma and the volume of myoma are evidenced. Since the dose applied is very low, toxicity is negligible. Application of this mixture gradually leads to menopause without disturbance of the hypothalamo-pituitary-gonadal axis. Application of this mixture does not provoke early or late complication specific for other medical approaches listed in the background of the invention. After completion of therapy with this medication, the volume of myoma is reduced by 20-30% and no surgical removal is required. After such therapy myoma does not grow any longer. After the therapy menstrual bleedings become normal in volume.

WO 03/022260 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

MEDICATION FOR TREATMENT OF UTERINE MYOMA

Description of invention

Field of invention

The invention belongs to the field of pharmacology and medicine i.e. gynecology, since it is a drug for conservative treatment of uterine myoma.

According to International Patent Classification (IPC) the invention is classified as: A 61 K 31/195.

Technical problem

The technical problem solved by the invention is the following: How to reduce the volume of uterine myoma, reduce the bleeding to a normal level without surgical intervention, without implementation of the existing therapeutic methods that are associated with numerous contraindications and, at the same time preserve all biological and sexual features of the female.

Myomas are the most commonly encountered benign tumors of the uterus. As many as 30-50% perimenopausal women suffer from myoma. Up to about 10 years ago the therapy was surgical and the problems of these women was managed by total hysterectomy with bilateral adnexetomy leading the patients to early menopause immediately.

Nowadays the following alternatives are available for the treatment of uterine myoma: progestagen, danazol, GnRH, embolization of myoma blood vessels by polyvinyl-alcohol. All these methods are associated with numerous adverse effects (disturbance of hypothalamo-pituitary-gonadal axis, abrupt onset of early menopause, necessitated anesthesia, inflammation, etc

This invention solves (cures) the uterine myoma disease without adverse effects that commonly accompany the current therapeutic methods by decreasing hemorrhage (which is the main symptom of uterine myoma), decreases the myoma volume and the patient, following the prescribed procedure, enters her menopause normally, without any surgical intervention.

Background of invention

In reference literature related to patents and other no treatment has yet been described to offer therapy of myoma of the uterus without contraindications associated with the known therapeutic methods that would make it possible for the patient to preserve all her biological and sexual features.

Current understanding of sickness and health as well as increasing need for prolongation of active sex life imply preservation of the internal sex organs. Conservative treatment of myoma of the uterus, thus, has become both challenge and need.

The therapy may be non-specific, when use of medication relieves the symptoms that result from the presence of myoma of the uterus and specific that in addition to relief of the symptoms also reduce the volume of the myoma of the uterus, uterine hemorrhage and improve hematological status of the patient.

In principle, pharmacotherapy of the myoma of the uterus can be used as monotherapy of supplementary. Medical therapy not only solves the problems of many patients, but frequently helps surgical therapy be less radical and safer for the patient.

The earliest conservative method for treatment of myoma of the uterus implied use of progestagen, whose role is to stop the proliferative changes in the endometrium, i.e. indirectly to stop abundant and prolonged uterine bleeding in women suffering from myoma of the uterus. This method is associated with ovarian cyst as the adverse effect, while the method itself only reduces the volume of bleeding while the myoma continues to grow.

Another method that is used with more or less success in order to reduce or stop heavy menstrual bleeding is use of danazol, antigonadotropin that reversibly influences the impact of gonadotropic hormones. Continuous application of these effectively reduces the volume of menstrual bleeding and brings the patient to the state of secondary amenorrhea, but the adverse effects are still numerous and dose related: hot flashes, heavy sweating, occurrence of acnae, hirsutism, weight gain and seborrhea.

GnRH analogues have been used for ten years now, and their continuous administrations irreversibly suppresses gonadal function leading to a condition similar to that hypogonadotropic hypogonadism. Long-term administration leads to discontinuation of estrogen secretion, resulting in discontinuation of menstrual bleeding, reduction and regression of myoma and improvement of the hematological status. Adverse effects include occurrence and signs of post-menopause: hot flashes and dry vagina encountered in over 90% patients, while insomnia and depression are also quite common. Early occurrence of

osteoporosis is the most serious adverse effect. Frequent adverse effects and associated risks are limiting factors of this therapy, while application of GnRH analogues disturbs the natural cycle of hormone secretion.

Vascular sclerozing of myoma of the uterus by alcohol is an effective non-surgical method aimed at selective sclerosing of the blood vessels that supply the myoma. Antibody sclerosis reduces supply to the myocytes, resulting in their degeneration with resultant necrosis and degeneration. The method is aggressive, associated with risk and may result in immediate complications.

Embolization of blood vessels is a method similar to the previously described one, where polyvinyl alcohol is injected into the myomal vessels to block them, preventing blood supply to the myoma vessels which may lead in aseptic necrosis and degeneration accompanied with sharp pain immediately after the procedure and occurrence of high fever.

The aim of the invention to reduce the volume of the myoma of the uterus, reduce the bleeding to a normal level, and avoid all the above complications and preserve biological and sexual features of the woman.

Summary of the invention

The invention is represented by a mixture of two substances.

The first substance is methotrexate: 4-amino-N-10-methyl-pteroilglutamic acid; CASRN: 59-05-2; $C_{20}H_{22}N_8O_5$; Molecular mass = 454.4444; insoluble in water (<0.1 g/100 mL at 19 ^{0}C); yellow to dark orange crystal powder; hygroscopic, sensitive to light. Methotrexate is a drug used for treatment of some malignant diseases, severe forms of rheumatoid arthritis and intact extrauterine pregnancy, hydatiform mole and choriocarcinoma.

The second substance is L-arginin; 2-amino-5-guanidino- pentanic acid; CASRN: 74-79-3; $C_6H_{14}N_4O_2$; Molecular mass = 174.2022; basic amino acid; essential amino acid; white powder.

Combination of two substances in aqueous solution yields a homogenous mixture where each of the components preserves its own chemical identity.

Local application of aqueous solution of the mixture of these two substances subendometrially into the myoma on the eighth day of the follicular phase of the menstrual cycle bleeding results in reduction of the myomal volume. The mixture is applied in three consecutive menstrual cycles. The method is simple, feasible on out-patient basis by

practicing gynecologist; no untrasonografic guidance is needed; transvaginal and transcervical approach is used. The mixture induces the process of apoptosis (programmed cell death) so that no necrosis, degeneration or inflammation are provoked if all antisepsis precautionary measures are applied. Dramatic reduction of both loss of blood during menstruation in women with myoma and the volume of myoma are evidenced. Application of this mixture gradually leads to menopause without disturbance of the hypothalamo-pituitary-gonadal axis.

The drug is most effective in treatment of myoma of the uterus with diameter up to 10 cm.

After completion of therapy with this medication, the volume of myoma is reduced by 20-30% and no surgical removal is required. After such therapy myoma does not grow any longer. After the therapy menstrual bleedings become normal in volume. The patient continues her normal life with preserved biological and sexual features until normal menopause when menstrual bleeding is discontinued and myoma of the uterus stops growing. The aim of the therapy is to bridge over the period to menopause with preserved biological and sexual features of the woman, i.e. without any adverse effects on her health.

Application of the drug does not induce any direct or indirect late complications specific for other medical treatments cited above.

Summary of the illustrations

Figure 1. illustrates molecular structures of methotrexate and L-arginine.

Figure 2. gives the illustration of molecular appearance of the homogenous mixture of methotrexate and L-arginine.

Detailed description of the invention

This pharmaceutical composition contains:

- 5 g methotrexate (CASRN: 59-05-2)
- 10 g L-arginine (CASRN: 74-79-3)
- 985 cm^3 water.

The first substance is methotrexate: 4-amino-N-10-methyl-pteroilglutamic acid; CASRN: 59-05-2; $C_{20}H_{22}N_8O_5$; Molecular mass = 454.4444; insoluble in water (<0.1 g/100 mL at 19 ^{0}C); yellow to dark orange crystal powder; hygroscopic sensitive to light. Methotrexate is a drug used for treatment of some malignant diseases, severe forms of

rheumatoid arthritis and intact extrauterine pregnancy, hydatiform mole and choriocarcinoma.

The second substance is L-arginin; 2-amino-5-guanidino- pentanic acid; CASRN: 74-79-3; $C_6H_{14}N_4O_2$; Molecular mass = 174.2022; basic amino acid; essential amino acid; white powder.

Figure 1. illustrates molecular structures of methotrexate i L-arginine.

Dissolution of methotrexate and L-arginine in water results in formation of homogenous mixture without any chemical reactions that might change the chemical structure of either of the components. However, some secondary interactions do take place in the homogenous mixture (Figure 2.) caused by acid-base reaction of methotrexate and L-arginine as well as the occurrence of hydrogen bond. L-Arginine as a very alkaline amino acid deprotonizes the carboxyl groups of methotrexate. The hydrogen bond between the methotrexate and L-arginine molecules is realized primarily between the oxygen atoms of the carboxyl groups of methotrexate and nitrogen atoms of L-arginine, as well as oxygen atom of the L-arginine carboxyl group and nitrogen atoms of methotrexate. Molecules of water are included in the network of the above hydrogen bonds.

Use the analytical balance (precision to the fifth decimal number) to weigh 10 g L-arginine and transfer quantitatively to a normal laboratory vessel of 1 dm^3. After that 600 cm^3 of re-distilled water is added to the vessel. Close the vessel and shake slowly until L-arginine is completely dissolved. Completely colorless solution is obtained. Use the analytical balance (precision to the fifth decimal number) to weigh 5 g methotrexate and transfer quantitatively to the laboratory vessel containing L-arginin solution. Shake the vessel mildly until methotrexate is completely dissolved. Fill to the volume mark with re-distilled water. Place the solution into standard autoclave for processing and store in refrigerator at 4 to 8 ^0C.

The single dose is 2 cm^3 of the drug solution in a 2 cm^3 syringe with a 20 G puncture needle. The set is sterilized.

The drug is most effective in treatment of myoma of the uterus with diameter up to 10 cm.

After completion of therapy with this medication, the volume of myoma is reduced by 20-30% and no surgical removal is required. After such therapy myoma does not grow any longer. After the therapy menstrual bleedings become normal in volume. The patient continues her normal life with preserved biological and sexual features until normal

menopause when menstrual bleeding is discontinued and myoma of the uterus stops growing. The aim of the therapy is to bridge over the period to menopause with preserved biological and sexual features of the woman, i.e. without any adverse effects on her health.

Mode of application

The drug is applied on the eighth day of the follicular phase of the menstrual cycle in patients with myoma of the uterus. The single dose is 2 cm^3 of the drug solution in a 2 cm^3 syringe with a 20 G puncture needle. The set is sterilized.

The patient is placed into lythotomy position, the vaginal speculum is placed and the uterine cervix is pulled with roller forceps so that the external uterine cervix is exposed. The package is open, the puncture needle is inserted into the cervical channel and is directed to the myometrium towards the side at which myoma is located. The needle is pushed through the endometrium to the depth of 1 cm. It is necessary to be sure to inject the drug at 1 cm depth to avoid possible penetration through the uterine wall. We aspirate to check that the needle is not inside a blood vessel, and after that inject the drug slowly. The syringe and needle are discarded after use. The procedure is repeated in three consecutive menstrual cycles.

The drug is most effective in treatment of myoma of the uterus with diameter up to 10 cm.

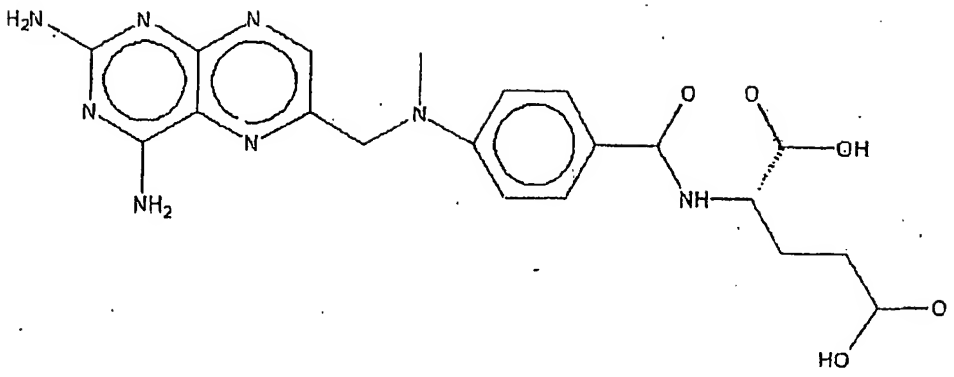
After completion of therapy with this medication, the volume of myoma is reduced by 20-30% and no surgical removal is required. After such therapy myoma does not grow any longer. After the therapy menstrual bleedings become normal in volume.

PATENT CLAIMS

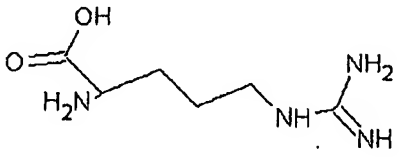
1. Product, aqueous solution of mixture of methotrexate and L-arginine ***wherein*** 5 g methotrexate and 10 g L-arginineare are mixed in 985 cm^3 of water.

2. Process of aqueous solution of the mixture production pursuant to this patent claim 1. ***weherein*** 10 g L-arginine is weighed on analytical balance and quantitatively transferred to a 1 dm^3 vessel where 600 cm^3 water is added, mildly shaken until L-arginin is completely dissolved. After that 5 g methotrexate is added, the vessel is mildly shaken until all methotrexate is completely dissolved, the remaining water is added, and the obtained solution is placed in a standard autoclave for processing.

3. The aqueous solution of the mixture is used, pursuant to patent claim 1 for treatment of myoma of the uterus.



Metotreksat



L-Arginin

Fig. 1

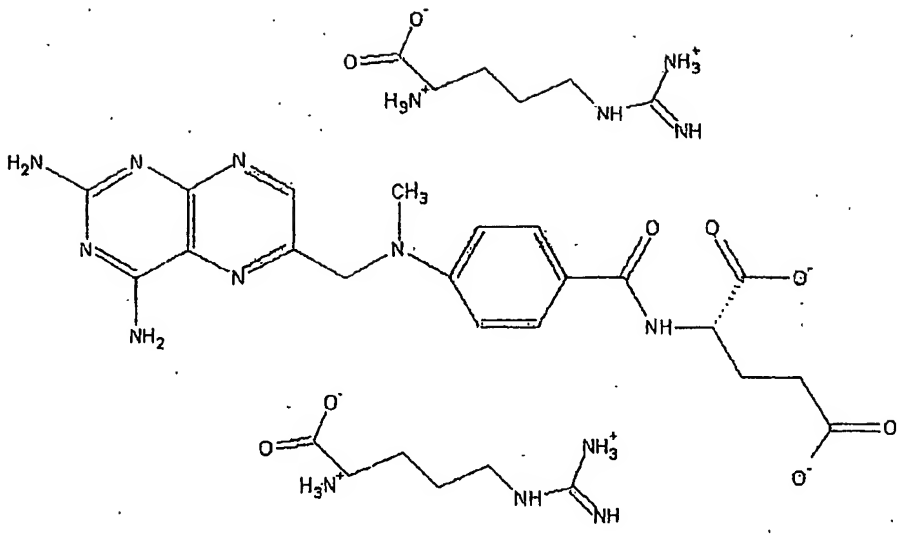


Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/YU 02/00017

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/195 A61K31/519 A61K9/08 A61P35/00 A61K31/505
//(A61K31/519,31:195)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, INSPEC, MEDLINE, EMBASE, WPI Data, PAJ, BIOSIS, PASCAL, CHEM ABS Data, SCISEARCH, CANCERLIT

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 202 445 A (MERCK PATENT GMBH) 26 November 1986 (1986-11-26) example 58 ---	1-3
A	EP 0 350 246 A (TAKEDA CHEMICAL INDUSTRIES LTD) 10 January 1990 (1990-01-10) page 3, line 8,54-57 ---	1-3
Y	ZIVANOVIC A. ET AL: "Methotrexat in the therapy of uterus leiomyomas." ARCHIVE OF ONCOLOGY, (1998) 6/3 (95-97). , XP001121815 abstract ---	1-3

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents:

'A' document defining the general state of the art which is not considered to be of particular relevance

'E' earlier document but published on or after the international filing date

'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 January 2003	21/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Zimmer, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NERI I ET AL: "Impact of the L-arginine/nitric oxide system in pregnancy." OBSTETRICAL & GYNECOLOGICAL SURVEY. UNITED STATES DEC 1995, vol. 50, no. 12, December 1995 (1995-12), pages 851-858, XP009003548 ISSN: 0029-7828 abstract	1-3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/YU 02/00017

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: –
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy Although claim 3 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/YU 02/00017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0202445 A	26-11-1986	DE 3513938 A1	23-10-1986
		AT 60904 T	15-03-1991
		AU 587432 B2	17-08-1989
		AU 5465686 A	23-10-1986
		CA 1282330 A1	02-04-1991
		DE 3677545 D1	28-03-1991
		EP 0202445 A2	26-11-1986
		ES 8801988 A1	01-06-1988
		HU 44170 A2	29-02-1988
		IE 57523 B1	07-10-1992
		JP 61243015 A	29-10-1986
		US 4797282 A	10-01-1989
		ZA 8602947 A	30-09-1987
EP 0350246 A	10-01-1990	AT 90557 T	15-07-1993
		CA 1339300 A1	19-08-1997
		DE 68907139 D1	22-07-1993
		DE 68907139 T2	02-12-1993
		EP 0350246 A2	10-01-1990
		ES 2055067 T3	16-08-1994
		HU 9500499 A3	30-10-1995
		JP 2124814 A	14-05-1990
		JP 2827287 B2	25-11-1998
		US 5271945 A	21-12-1993

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 March 2003 (20.03.2003)

PCT

(10) International Publication Number
WO 03/022260 A1

(51) International Patent Classification[7]: A61K 31/195, 31/519, 9/08, A61P 35/00, A61K 31/505 // (A61K 31/519, 31:195)

(21) International Application Number: PCT/YU02/00017

(22) International Filing Date:
10 September 2002 (10.09.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
P-657/01 12 September 2001 (12.09.2001) YU

(71) Applicants and
(72) Inventors: **ARSENIJEVIC, Slobodan** [YU/YU]; Mite Cekica 22, YU-Kragujevac (YU). **MATOVIC, Zoran** [YU/YU]; Kragujevackog oktobra 145/11, YU-Kragujevac (YU).

(74) Agent: **KOJIC, Dragomir, M.**; Kneza Milosa 43, YU-Belgrade (YU).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

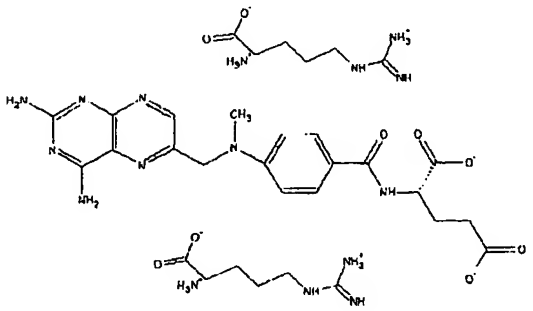
Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
with international search report
with amended claims

Date of publication of the amended claims: 16 October 2003

[Continued on next page]

(54) Title: USE OF METHOTREXATE AND L-ARGININE FOR THE PREPARATION OF A MEDICAMENT FOR TREATMENT OF UTERINE MYOMA



(57) Abstract: The invention illustrates a drug for treatment of myoma of the uterus that enables preservation of biological and sexual properties of the women and does not provoke contraindications accompanying other known methods of treatment. The invention is represented by a mixture of two substances methotrexate and L-arginine. The drug for treatment of myoma of the uterus is composed of 5 g methotrexate and 10 g L-arginine with 985 cm^3 water. Combination of two substances in aqueous solution yields a homogenous mixture where each of the components preserves its own chemical identity. The drug is applied on the eighth day of the follicular phase of the menstrual cycle of patients with myoma of the uterus. The patient is placed into lythotomy position, the vaginal speculum is placed and the uterine cervix is pulled with roller forceps so that the external uterine cervix is exposed. The package is open, the puncture needle is inserted into the cervical channel and is directed to the myometrium towards the side at which myoma is located. The needle is pushed through the endometrium to the depth of 1 cm. It is necessary to be sure to inject the drug at 1 cm depth to avoid possible penetration through the uterine wall. We aspirate to check that the needle is not inside a blood vessel, and after that inject the drug slowly. The syringe and needle are discarded after use. The mixture is applied in three consecutive menstrual cycles. The method is simple, feasible on out-patient basis by practicing gynecologist; no ultrasonografic guidance is needed; transvaginal and transcervical approach is used. The mixture induces the process of apoptosis (programmed cell death) so that no necrosis, degeneration or inflammation are provoked if all antisepsis precautionary measures are applied. Dramatic reduction of both loss of blood during menstruation in women with myoma and the volume of myoma are evidenced. Since the dose applied is very low, toxicity is negligible. Application of this mixture gradually leads to menopause without disturbance of the hypothalamo-pituitary-gonadal axis. Application of this mixture does not provoke early or late complication specific for other medical approaches listed in the background of the invention. After completion of therapy with this medication, the volume of myoma is reduced by 20-30% and no surgical removal is required. After such therapy myoma does not grow any longer. After the therapy menstrual bleedings become normal in volume.

WO 03/022260 A1

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMENDED CLAIMS

[Received by the International Bureau on 20 March 2003 (20.03.03):
original claims 1-3 modified [1 page]

1. A method for producing aqueous solution of methotrexate (4-amino-N-10-methyl-pteroilglutamine acid) and of L-arginine (2-amino-5-guanidine-pentane acid), **characterized in that,** a measured quantity of 10 g of L-arginine in a vessel of 1 dm^3 of volume is added a 600 cm^3 of water, where the vessel is softly shaked until the L-arginine is completely dissolved, after when 5 g of methotrexate is added and vessel softly shaked again until all methotrexate is dissolved, whenafter is in the vessel poured a remnant amount of 360 cm^3 til 390 cm^3 of water, and a so obtained solution than autoclaved in a tandard autoclave.

2. Pharmaceutical preparation made of aqueous solution of a mixture of methotrexate (4-amino-N-10-methyl-pteroilglutamine acid) and of L-arginine (2-amino-5-guanidine-pentane acid) obtained by method according to claim 1, **characterized in that,** in a 985 cm^3 of water is mixed a 5g of methotrexate and 10 g of L-arginine.

3. Use of a methotrexate (4-amino-N-10-methyl-pteroilglutamine acid) and of L-arginine (2-amino-5-guanidine-pentane acid) for manufacture of medicine for reduction of volume of myoma uteri.